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Reproductive cycle of female Pit Viper (*Gloydius halys caucasicus*) in Iran

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Abstract

The data on reproductive cycle of the Caucasian viper (*Gloydius halys caucasicus*) from cold mountains of the Iran is presented here in. Knowledge about the reproductive cycle is an important tool for assisted reproduction and is prerequisite for animal breeding in the captivity. Reproductive parameters of 24 female Caucasian snakes from Takht-e-Solaiman mountain-Taleqhan (Cold Mountain), Alborz province in central Iran were studied during different months of year, 2013. The reproductive tracts of samples were examined and follicular developmental stages were assesed according their size and the embryo presence and development were evaluated under stereomicroscope. The follicles were grouped in four types including; types I (2.5-3.3 mm) and type II (5.7-8.4 mm) that were observed in the ovaries from July to November. The number and percentage of follicles type I were maximum in November, 80% (n=28) in the right ovary, and 81% (n=27) in the left one, and these values for follicles type II were maximum in October, in right ovary, 27% (n=13) and left one, 31%

(n=14). Ductal follicules were types III (20.5- 22.9 mm) and IV (>29 mm) and observed from July to September. The number and percentage follicules III were maximum in july, in right and left ducts were respectively 12%(n=2) and 8%(n=7) and these values for follicules type IV that were maximum in the September, in right and left ducts were respectively 20%(n=10), and 16%(n=7). The gestation time was from mid-July to late September, lasted for 2.5 months. It seems that mating in this snake occurs in June and the Iranian Pit Viper may have biennial reproductive cycle that requires more study.

Keywords: adult viper- Iranian pitviper - reproductive cycle - follicular growth

Introduction

The study of biology of reproduction and reproductive cycle provides valuable information for animals breeding and preservation. The present study regarding the reproductive characteristics of *Gloydius halys caucasicus* (locally called Caucasian viper) is conducted in order to determine the reproductive potential of this biomedical and economically important snake of the Iran. *Gloydius halys caucasicus* is a venomous, viviparous snake, generally spreaded in the mountainous areas, geographically in the southwest and southeast of the Caspian Sea, south east of Azerbaijan, south of Turkmenistan, former Soviet Union, north of the Iran and far northwest of Afghanistan. The Caucasian vipers' distribution in Iran is reported in the Tehran, Alborz, Gilan, Mazandaran, Golestan, North Khorasan, Khorasan and Semnan provinces (13, 28). This snake is used in serum and vaccine inistitutes to produce biological products such as venom and antivenin against the snake bite. The snake venom contains various enzymes that are used in the biochemical/ medical researches, medical and therapeutical purposes (20, 16).The reported data are mostly on the mechanism of action and effect of venoms, some informations are about the biosystematics (28) and the internal systems of the snakes (13) and so far, no data has been reported about the reproductive cycle of the female Caucasian viper of the Iran. It is well known that the ovarian follicular growth, formation of the mature egg, fertilized egg, and finally the formation of embryos, are representing different stages of the reproductive events in the viviparous snakes(3). In order to study the follicular growth stages, the researchers have grouped the ovarian and ductal follicles of the snakes based on the follicular size, into different types and sizes. Kofron (1979) has reported four group of follicular developmental stages in the female Brown snake,

Storeria dekayi, from Louisiana, including; group 1: 0.1-1.5 mm, immature follicles; group 2: 1.6-3.0mm, follicles have been achieved from the first group of follicles; group 3: 6.1-12.0 mm, follicles contain yolks that are usually mature in the late winter and early spring; group 4: 6.1-12 mm, follicles indicate the mature female snake (27). According to Tasi and Tu (2001), reproductive development in the mature female snakes was classified in the four stages (32, 8), The First type or primary follicles have length less than 5mm. The second type or secondary follicles have the length of 5-15 mm. Third type follicles have length of 15-25 mm. Fourth type are follicles with more than 25 mm length.

It is reported that there are some differences in mating time and the vitellogenesis seasons in the female vipers from different zones (Beaupre and Duvall, 1998). Although, in the most of the snakes, vitellogenesis starts in the spring, following emerging from hibernation and reach to the peak at the time of ovulation. In some species of temperate zone viper like *C. atrox,* vitellogenesis begins in the spring (vitellogenesis type I) (2). In the female *Bothrops insularis* viper, vitellogenesis occurs in the spring and the females ovulate in the late spring. In some other vipers, vitellogenesis begins in the summer or fall, the follicular activity stops throughout the winter and continues and completes in the spring and early summer (vitellogenesis type II) (Beaupre and Duvall, 1998). Almeida and Saloma (2002) also reported that in some of the female pit vipers from temperate regions, ovulation begins at the end of summer and stops during the hibernation, then once again starts in the spring at beginning of the second mating season and continues till the ovarian follicles to be prepared to ovulate. In the case of fertilization, few embryos are found in the uterus until parturition that occurs at the end of the next summer and beginning of autumn, following the first summer / fall mating (4).

In other species, the reproductive cycle (ovulation and parturation) is in such a way that the females ovulate at the beginning of the summer and give birth to offsprings at the end of the summer (3).

Three mating patterns are reported in the pit vipers from temperate regions (33). Spring pattern; breeding / mating is only in the spring. Males have to store sperms in the vas deferens (excretory ducts of the testicles) all over the winter till vernal breeding season. The species with spring reproduction pattern are only the pit vipers that their spermatogenesis is completely disociated from mating.

Fall pattern; breeding / mating is at the end of the summer and autumn. The reproductive season at the end of the summer and fall is only the pattern that is associated with

spermatogenesis that is common in the most of the pit vipers from temperate areas (3). In this species the long term sperm storage (LTSS) is compulsory (35). Bimodel spring and autumn pattern; breeding / mating occurs in the two seasons (3). The snakes mate in spring and autumn; it is commone in the vipers from the temperate climates such as *A.conturatus*, *C.atrox, C.cerastes and C.horridus*. This matting pattern may be related to the fall spermtogenisis and independent of the spring spermatogenesis. These species have optional LTSS, it means the females either use sperms of fall mating that is stored during winter or sperms of the spring mating, or the sperms of the both seasons (36). The factors determining which sperms (sperm of the fall or spring mating) to be use to fertilize the eggs during ovulation in the summer are not known.

Since there are variations in the reproductive cycle patterns of different vipers, this study is conducted to provide data on the reproductive cycle of the *Gloydius halys caucasicus* of the cold mountain of the Iran as prerequsite for captive breeding.

Materials and Methods

A total of 24 females *Gloydius halys caucasicus* (20 mature females and 4 pregnants snakes) were collected from Takht-e-Soleiman in Taleghan, Alborz province, Iran during year 2013. Snakes were kept in the conventional vivariums. The pregnancy was esured by palpation of the snake's belly. After weighing animal with scale, body length from snout to vent (SVL) and tail length from cloacae to the end of tail were measured by the calibrated tape (7). In order to study the reproductive parameters, snakes were sacrificed by injection (sc) of 1% lidocaine (20 mg kg^{-1}, Daroupakhs, Iran) around cloacae. All procedures were carried out according ISIRI 7216-2 animal ethics guidelines (24) Reproductive tract and ovaries were removed and placed in a Petri dish containing PBS and different parts of the duct were examined under stereomicroscope and follicular lenght was measured by micrometer.

Results

The large mature females *Gloydius halys caucasicus* were weighed 78.33 ± 15. 38 g and the small ones, 37.5 ± 8.6 g, the maximum mean of SVL was 44.66 ± 3.43 cm (mean tail length, 5.41 ± 0.86 cm) and the minimum mean of SVL was 38.5 ± 2.3 cm (mean tail length, 4.41 ± 1.3 cm). Pregnancy months were in July, August and September and the parturition has

occured in the late September. The mean number and percentage of the ovarian and ductal follicles (according their length) in mature female *halys caucasicus* in different monthns are shown in Table 1, and embryonic developmetal stages are presented in Figure 1.

When the follicles size reach to their maximum length, infundibulum grows wider and longer. Ducts sections are clearly diagnosed from each other, uterine glandular, non-glandular uterus and vagina are also completely recognizable as the tissue of the glandular uterus to the non-glandular uterus was distinctly darker. The vagina is clearly discernible during ovulation and gestation.

Table 1: Mean number and percentage of ovarian (types I & II) and ductal follicles (types III & IV) in *Gloydius halys caucasicus* of Iran in different months of the year

Type / Months	Number and types of ovarian follicles				Number and types of ductal follicles			
	Right ovary		Left ovary		Right duct		Left duct	
	I 2.5-3.3 (mm)	II (5.7-8.4 mm)	I (2.5-3.3 mm)	II (5.7-8.4 mm)	III (20.5- 22.9 mm)	IV (>29 mm)	III (20.5-22.9 mm)	IV (>29 mm)
July n=4	65.09% n=69	11.32% n=12	62.5% n=50	12.5% n=10	12.26% n=13	11.32% n=12	8.75% n=7	16.25% n=13
August n=4	61.90% n=65	14.28% n=15	68.18% n=60	11.36% n=10	10.47% n=11	13.33% n=14	7.95% n=7	12.5% n=11
Sept. n=4	47.91% n=23	27.08% n=13	57.14% n=24	21.42% n=9	4.16% n=2	20.83% n=10	4.76% n=2	16.66% n=7
Oct. n=4	73.77% n=45	26.22% n=16	68.88% n=31	31.11% n=14	–	–	–	–
Nov. n=4	80% n=28	20% n=7	81.81% n=27	18.18% n=6	–	–	–	–

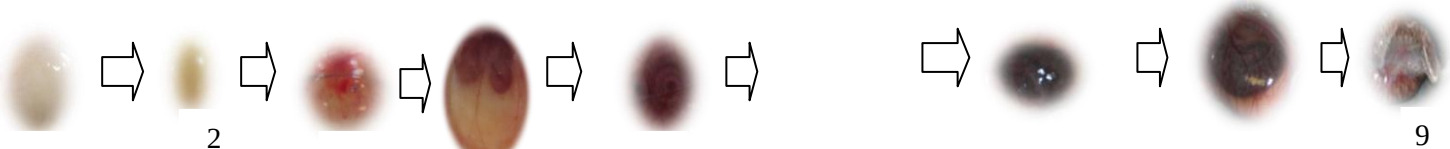

Figure 1: Embryo developmental stages from follicular growth to complete embryonic growth, 1- mature follicle, 2- Pre ovulatory follicle, 3- Fertilized egg (late Jun), 4- Formation of early embryos (July), 5-6-7-8- Embryo development (August). 9- Complete fetus (September).

Discussion

Distribution of vipers in the different areas with different climates, results to the varieties in thier seasons of activity (23, 22) that naturally affect the reproductive cycle and breeding potential (20, 21). The ovarian follicular growth, formation of the mature egg, fertilized egg, and finally the formation of embryos, are representing different stages of the reproductive events in the viviparous snakes (3). In the present study, the follicules type I and II were present through July to November and percentages of these follicles are maximum in November. The follicles larger than 10 mm in female snakes were observed at the beginning of July, it shows that the vitlogenesis cycle starts at the late spring or early summer. Progress of follicular growth is continued during the active season and may have stopped during hibernation in the winter and resumed the following spring. Since the large number of the ductal follicles, type III and IV are observed in the early summers (June), ovulation may have occured from May (spring) to July (early summer). However, the early embryos were observed in mid-July and developed embryos, in late August, embryos reached to their full growth in the September, and parturation has occurred at the end of September (summer). So during the summer from mid-June to late September is considered as gestation period (2.5 months). Due to the occurrence of ovulation in May (spring) and pregnancy of females in mid-July, the mating/ fertlization may have occured at the time of ovulation in late spring or early summer. Mozafari et al., (2012) have reported Iranian Caucasian male viper of Iran like clobrides of mid-temperate zone have summer spermatogenesis (25, 33). In the snakes, mating, ovulation and fertilization usually occurs at a time of year when environmental conditions like temperature, food availability are good for reproduction and breeding (16). In the female *vaciolar* from the mid-tempreture zone of United States, ovulation occurs in early summer and offsprings are born at the late summer (4). In *the Storeria dekayi* from the Louisiana area, yolk formation in the most of the females is in the early spring (April), ovulation occures during April and pregnancy lasts 2.5 months and birth occurs during the second half of June and July. In the *N.f. confluens* of southeast area in Louisiana, the follicular growth begins in early May, followed by mating and growth of follicles in spring and

reproductive cycles of the snakes is influenced by weather conditions and similar to the clobrides of mid-tempreture zone having spring vitelogenesis and summer Spermatogenesis (33). In the Vipera lataslei in the north of Portugal, ovulation occurs in the late May to early June, and newborns are observed in the early August (10). It is also reported that the female Vipera lataslei have at least a biennial reproductive cycle. Mating in Vipera lataslei is limited to late summer or fall (28) and spring mating is absent or very low. This result is unexpected because the spring is usually the most common European viper mating period (25, 26). In the *Vipera aspis* in the west of France, ovulation is in spring (April to June), and birth is reported in late August (9). Distribution of Crotalus viridis is in the United States and Canada and the most varieties are in North of America. They are generally active in April to September and mating occures in late spring and summer coinciding with the peak of spermatogenic activity. It is thought that *Crotalus darissus* has a biennial cycle, but annual cycles are also annonced. Season of birth lasts from late summer (August) to early autumn (October) (13). The *Crotalus darissus* of northeastern Brazil and other species of Crotalus in the mid-temperate zone show similar reproductive cycle (5, 6). Spermatogenesis begins in the spring and reaches to its peak in summer (27). Sperm stores in the vas tube until the ovulation in the fall. Fertilization and ovulation occurs not coincide with mating. Vitelogenesis begins in the fall and continues untill winter up to the time of ovulation and pregnancy in the spring. Early vitelogenesis exists whole the year but secondary ones is from end of spring to end of fall (July and August). Pregnancy occurs in the end of spring during summer and ovulation and mating are not simultaneously. The Crotalus lutosus in America that exists in the the cold desert (32) and the hot desert that vitelogenesis in the cold desert vitelogenesis occurs in late spring and early summer (24) and in the hot desert vipers, vitelogenesis occurs in summer or autumn. Many *Crotalus lutosus* ovulate in late spring (19). Study on the morphology of reproductive of female *Agkistrodon piscivorus* is an interesting opportunity to test biennial reproductive cycle that observed in this spiece of female snakes (15). In the pythons is also seen in other mid-temperate zone (7). In the female pythons in mid-temperate climate, vitelogenesis begins from late summer to early autumn. Sperm appears in the snake's fallopian tube at this time (17, 29). Ovarian follicular continue its growth until ovulation in late spring. Females are pregnant until late summer / fall, pregnant snakes have fetus until delivery and the birth occurs in late summer and autumn and the spring is breeding season (11). *Boiga irregularis* from tropical and subtropical regions of the eastern islands of New Guinea and northwest Mlanzya to the east coast of Australia. Ovulation occurs in the fall (October and November). Vitelogenesis occurs in September and October. Storage of sperm in the fallopian tube has not been

reported. In Rhinocheilus lecontei of southeastern United States and eastern north Mexico (30), it is thought that mating occurs in the spring after a winter of inactivity. Reproductivity apparently occurs in the spring, although there was sperm in vas deferens tubes during all tested snakes. There were female snakes with ovarian follicular larger than 12mm in length from April –July. Female snakes with producing secondary yolk in April - May (14). Since spermiogenesis occures during early summer (July) and fall (October), male snakes are subject to a seasonal cycle of sperm production in July and October.

Crotalus durissus terrificus exists in southeastern Brazil. Seasonal reproductive activity in southeastern Brazil is in late summer (March-February) to early winter (June, July) (27). Spermatogenic activity begins in spring and in summer, sperm production reaches to its peak, so its reproductive cycle pattern is after mating (28). In the cycle, sperm storage is essential. In the snakes, vitelogenesis and mating are simultanously in the fall (5). Female snake stores sperm during the fall and winter in the womb and fertilization occurs in the spring. The snake has an annual cycle (6). Ovarian follicles may vitellogenize during spring that the development and enlargement of follicles (8, 21) have been reported. In *Gloydius halys caucasicus*, ovarian follicles (types I and II) were observed in May, and ductal follicles (types III and IV) in June after ovulation, probably ovulation time is from late May to early July and pregnancy time occurs from mid July to late September and parturition occurs in the late September. It seems that mating in the snake occurs in June and reproductive duration in *halys caucasicus* may be biennial which requires further studies.

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